Fighting fungi with physics: Food wrapping by a solitary wasp prevents water condensation

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Mold contamination of stored food is a hazard shared by humans and many animal species. It inevitably leads to the loss of laboriously gathered and exigently needed alimentary supplies and should thus be avoided by all means. Here, we report on a mechanism employed by a solitary wasp to preserve its larval provisions against molding. Female European beewolves, *Philanthus triangulum* (Hymenoptera: Crabronidae), hunt honeybees and store one to six paralyzed bees in subterranean brood cells as food for each of their larvae. Prior to egg-laying they apply large amounts of a secretion from a postpharyngeal gland to the surface of the bees. This has a surprising physical effect: it heavily reduces the accumulation of water on the provisioned bees. Embalming by beewolf females covers microstructures on the bee surface that might otherwise function as nucleation sites for water condensation. Crucial to this effect is that it increases the proportion of unsaturated hydrocarbons on the prey surface. The prevention of water condensation renders the microclimatic conditions unfavorable for the development of mold fungi. This physical preservation mechanism represents a novel adaptation of higher animals in their combat with fungi.

The humid and warm conditions in beewolf nests constitute an ideal environment for the germination and fast growth of mold fungi [1,2]. Hence, the prey has to be preserved to prevent molding during the eleven-day larval period. Several insect species have evolved chemical secretions that inhibit fungal growth [3,4]. The beewolf secretion clearly delays fungus growth on bees [1]. However, inhibition zone assays show that this is not due to a direct antimycotic effect (Herzner *et al.*, in press). Antibiotics produced by recently discovered symbiotic bacteria of the beewolf cannot be involved as these bacteria are not present on the bees [2]. We therefore explored possible non-chemical effects of the postpharyngeal gland secretion taking into account the well known Achilles' heel of mold fungi, their dependence on humid microclimates [5].

The prey embalming by beewolf females increased the total amount of hydrocarbons on the bees about tenfold (unembalmed: 8.1 ± 4.4 μg; embalmed: 78.6 ± 4 3.7 μg; exact $p < 0.0001$). Scanning electron microscopy revealed that embalmed bees were completely 'wrapped' by the secretion, which appears to form a thick and contiguous coating (Figure 1B). Microstructures such as the sockets of antennal sensilla and tiny contaminants (Figure 1A) were completely covered. Such structures are known to promote water condensation and infectious processes by fungi [6–8]. Besides these structural changes, the physico-chemical properties of the prey's surface might be changed. The postpharyngeal gland secretion consists primarily (78.8%) of unsaturated long-chain hydrocarbons. It thus differs from the typical cuticular hydrocarbon layer of insects, which mainly consists of saturated and methyl-branched hydrocarbons. Consequently, the proportion of unsaturated hydrocarbons is about threefold higher on embalmed (73.6 ± 9.9%) as compared to unembalmed bees (25.8 ± 7.8%, exact $p < 0.0001$). Such an increase in the proportion of unsaturated hydrocarbons on the cuticle reduces the melting point of the total hydrocarbon mixture [9,10]. It becomes more or less liquid and forms an oily, hydrophobic layer.

To test if these structural and physico-chemical changes of the prey surface reduce water condensation, we kept embalmed and unembalmed bees under moist conditions (26–29°C at 100% relative humidity). After 12 hours, the surface of unembalmed bees was covered with countless water droplets (Figure 1C), while embalmed bees appeared almost dry (Figure 1D). The embalming heavily reduced the

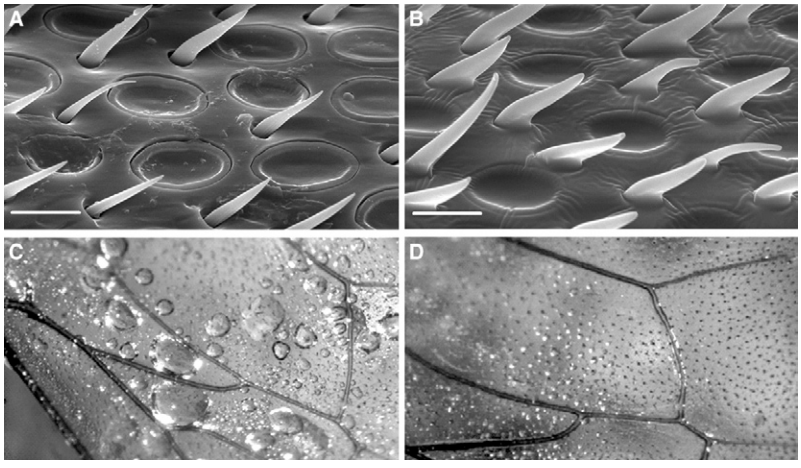

Figure 1. Microscopic effects of bee wrapping by beewolves.

(A) Scanning electron microscopy picture of a normal honeybee antenna (scale bar: 10 μm). Note the clearly visible furrows and grooves surrounding the oval-shaped pore plates and cuticular hairs as well as small dirt particles. (B) An embalmed honeybee antenna (scale bar = 10 μm). The surface is coated with a contiguous layer of secretion. The grooves surrounding the pore plates are sealed and the dirt particles covered by the secretion. The folds may be artifacts caused by the high vacuum conditions in the SEM. (C) Forewing of an unembalmed honeybee after three days in an artificial brood cell under moist conditions (Supplemental data). Large drops of water have formed due to condensation on the surface. (D) Forewing of an embalmed honeybee after three days in an artificial brood cell under moist conditions. The surface is almost dry.

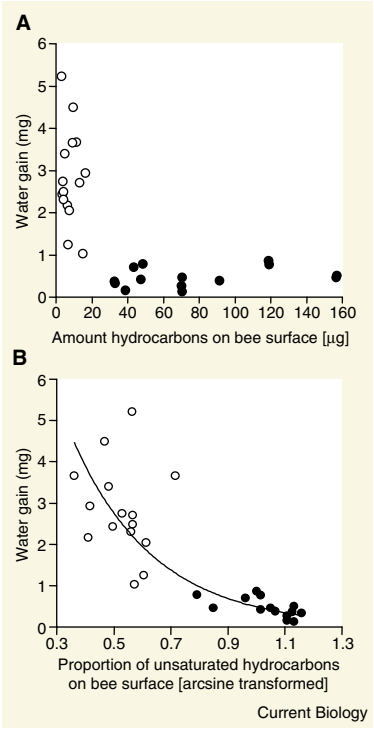

Figure 2. The influence of the surface hydrocarbons on water accumulation.

Unembalmed bees (white circles) were compared to embalmed bees (black circles). (A) The quantity of water gained in the experiment as a function of the amount of hydrocarbons. (B) The quantity of water gained in the experiment as a function of the proportion of unsaturated hydrocarbons (exponential regression: n = 29, $r^2 = 0.82$, $p < 0.001$).

accumulation of water on the bees (0.47 ± 0.23 mg *versus* 2.8 ± 1.12 mg in unembalmed bees, exact $p < 0.0001$).

This effect on water condensation seems to depend more on qualitative than on quantitative changes on the bee surface (Figure 2). About 30 µg of hydrocarbons were sufficient to prevent water condensation on embalmed bees. While this is only slightly more than the maximum amount of hydrocarbons on unembalmed bees, the difference in the accumulation of water is profound. By contrast, increasing proportions of unsaturated hydrocarbons caused a continuous exponential decline of the amount of water. The unsaturated hydrocarbons seem to be responsible for the high film-forming properties of the secretion, allowing for a contiguous, effective coating.

The observed reduction in water condensation might be of great ecological relevance for beewolves. In the moist brood cells, bees would be at risk of molding, as they accumulate water due to daily temperature fluctuations of about 8°C and condensation [1,5]. We tested the effect of the embalming on mold growth and found a highly significant delay both in the onset of fungus growth (Cox regression, n = 67; $\chi^2 = 6.81$, df = 1, $p = 0.009$) and in the formation of spores ($\chi^2 = 16.18$, df = 1, $p < 0.001$) on embalmed bees as opposed to unembalmed bees (Supplemental data). At the end of the experiment, all unembalmed bees were completely covered by white or yellow-green spores, whereas embalmed bees never developed such dense mold covers.

Finally, we tested whether the decrease in molding could be caused by reduced water condensation. When we prevented water condensation on unembalmed bees, they never molded (Supplemental data). Moreover, when embalmed bees were sprayed with water to simulate water condensation, spore formation occurred significantly earlier than on embalmed, unsprayed bees ($n = 28$; $\chi^2 = 5.44$, df = 1, $p = 0.02$; Supplemental data), although the onset of fungus growth did not differ. At the end of the experiment, the sprayed bees were as heavily overgrown by mold as unembalmed bees under moist conditions. However, spores formed later on wet embalmed bees than on wet unembalmed bees (Supplemental data). Thus, the protective effect of the embalming seems to go beyond the reduction of water condensation. Nonetheless, the mere presence or absence of water on the bees was an important determinant for the development of molds.

We, thus, conclude that the prevention of water condensation mediated by the wrapping of the prey bees with the postpharyngeal gland secretion can considerably impair the growth of mold fungi. Such physical mechanisms against the growth of microbes might be much more widespread than currently known and might provide a basis for technical applications.

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Supplemental data

Supplemental data containing experimental procedures, figures and a movie are available at: http://www.current-biology.com/cgi/content/full/17/2/R46/DC1

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